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Original article

Colour variability of beef in young bulls from fifteen European breeds

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Summary The objective of this study was to determine the variation of the colour of *longissimus thoracis* muscle within and among fifteen European cattle breeds reared under comparable management conditions. A total of 436 unrelated purebred young bulls from fifteen European breeds (Aberdeen Angus, Highland, Jersey, South Devon, Danish Red, Holstein, Simmental, Asturiana de las Montañas (also known as Casina), Asturiana de los Valles, Avileña-Negra Ibérica, Pirenaica, Marchigiana, Piemontese, Charolais and Limousin) were reared in five experimental research centres in the United Kingdom, Denmark, Spain, Italy and France. The pH of M. *longissimus thoracis* was measured at 24 h and after 10 days of ageing and colour at 48 h and 10 days. Two generalised linear models, Pearson correlations and a hierarchical cluster analyses were carried out. Lean meat colour differed significantly between breeds, and these fifteen European breeds could be grouped according to four classes of commercial interest: 'very bright and pale-red', 'bright and pale', 'red' and 'dark and dull red'. These groups were partially related to body size and carcass traits, fatness and muscle development and structure, and were controlled by differences in gene expression within each breed.

Keywords Cattle, cluster, colour difference, loin, pH.

Introduction

Consumer preference and characteristics of meat available in each country or market, determines the level of meat consumption. Colour is a major intrinsic trait that influences the consumer's intention to purchase (Lynch *et al*., 1986), as they relate colour to freshness and the sensory eating quality. In the Mediterranean European countries, consumers show a preference for pink or pale-red coloured beef, while in the UK or Germany consumers like red meat (Corcoran *et al*., 2001). However, the colour of fresh meat is not well correlated with the eating quality (Taylor, 1996). To evaluate colour is a complex task because meat colour evaluation is a subjective assessment. Meat colour is affected by several factors, including breed and pH (Ripoll *et al*., 2012). The relation between meat colour and pH is widely accepted, especially the effect on b* and hue angle of beef (Mancini & Hunt, 2005). A large number of genetically distinct cattle breeds exist in Western Europe and this genetic diversity produces meat with many different quality traits (Albertí *et al*., 2008). In the European market, beef carcasses are valued on the basis of animal category (bull, steer, heifer, cow), carcass weight and the European carcass classification scores which is based on conformation and fat cover (E.U., 2008). However, this assessment has no

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relationship with the eating quality of beef (Bonny *et al*., 2016). New indicators have been proposed to improve the SEUROP classification such as meat colour (Monteils *et al*., 2017). Today, measurement of meat colour is not compulsory, and in the voluntary labelling (E.U., 2000) of colour assessment is subjective using colour reference standards. Many studies have found significant differences between breeds in colour traits, but the difficulty arises when the CIELab have to be interpreted as commercial colour differences. The present study examines variation of the colour of *longissimus thoracis* muscle within and among fifteen European cattle breeds reared under comparable management conditions.

Material and methods

Animal and rearing conditions

All procedures were approved by the in-house Ethics Committee for Animal Experiments of each participating research centres. The care and use of animals were in accordance with the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes (E.U., 2010).

A total of 436 unrelated pure breed young bulls from fifteen European breeds were reared in five experimental research centres in the United Kingdom, Denmark, Spain, Italy and France. The breeds included in the study were: Aberdeen Angus, Highland, Jersey, South Devon, Danish Red, Holstein, Simmental, Asturiana de las Montañas (also known as Casina), Asturiana de los Valles, Avileña-Negra Ibérica, Pirenaica Marchigiana, Piemontese, Charolais and Limousin. The number of animals per breed is shown in Table 1. Animals were selected to be as unrelated as possible to ensure that the full range of genetic diversity present within breeds was included in the study.

A uniform beef management system, representative of those used in European Union countries, was used for all breeds to homogenise as far as possible the influence of management and rearing systems on meat quality. All the animals were transported to the experimental farms at 9 months of age. Then, they were divided into groups of seven to eight animals and fed a standardised diet. The diet consisted of a concentrate compounded from barley flakes (80–84%), soya bean meal (7.5–11%) sodium bicarbonate (0.6%) with suitable vitamin supplements (1.5%) and barley straw, all fed *ad libitum*. The energy density ratio ranged from 12.9 to 13.5 ME/kgDM. The protein content was 160 g CP/kgDM up to 10 months of age and then decreased to 150 g CP/kg DM to slaughter. The space available to the animals was approximately 9 m^2 per animal. Performances, body size and carcass characteristics of the fifteen breeds were reported by Albertí *et al*. (2008).

Table 1 Number of animals, slaughter weight and age, and pH at 10 days from fifteen European young bulls breeds

Breed	*n*	Slaughter weight (kg)	Slaughter age (d)	pH 10 days
Aberdeen Angus	30	597.7 ± 4.6^{bcd}	428.6 ± 8.8^{cd}	5.63 ± 0.01^{abcd}
Asturiana de los Valles	30	557.7 ± 8.8^{ef}	460.6 ± 5.5^{b}	5.57 ± 0.01^{bcde}
Avileña-Negra Ibérica	30	550.9 ± 13.4^{efg}	462.2 ± 6.3^{b}	5.57 ± 0.01^{bcde}
Casina	31	443.5 ± 7.1^{h}	461.4 ± 4.7^{b}	5.59 ± 0.01^{abcde}
Charolais	30	634.0 ± 7.3^{a}	460.6 ± 3.9^{b}	5.57 ± 0.01^{bcde}
Danish Red	29	580.0 ± 10.6^{cde}	454.3 ± 2.8^{b}	5.58 ± 0.01^{abcde}
Highland	29	443.5 ± 3.4^{h}	510.6 ± 8.9^{a}	5.65 ± 0.02^{abc}
Holstein	29	596.3 ± 9.3^{bcd}	458.0 ± 1.0^{b}	5.64 ± 0.03^{abc}
Jersey	31	378.4 ± 1.5^{i}	414.7 ± 6.4^{de}	5.68 ± 0.02^{ab}
Limousin	31	565.4 ± 5.4^{ed}	428.0 ± 4.1^{cd}	5.56 ± 0.01^{cde}
Marchigiana	28	523.5 ± 7.2^{g}	459.2 ± 3.7^{b}	5.52 ± 0.01^{de}
Piemontese	30	527.3 ± 7.3^{fg}	461.0 ± 3.5^{bc}	5.51 ± 0.01^{e}
Pirenaica	31	602.4 ± 9.5^{abc}	444.8 ± 5.8^{bc}	5.54 ± 0.01^{cde}
Simmental	20	621.8 ± 20.9^{ab}	455.9 ± 2.4^{b}	5.69 ± 0.03^{a}
South Devon	27	591.7 ± 6.2^{bcd}	398.5 ± 9.0^{e}	5.60 ± 0.01^{abcde}

Means ± standard error.
Different lowercase letter in the same column implies statistical differences between breeds ($P < 0.05$).

Sampling and measurements

At approximately 75% mature bull weight, animals were slaughtered by captive bolt pistol and exsanguination in either commercial or experimental slaughterhouses, depending on the experimental facilities of each country. Carcass dressing followed a standardised project protocol without use of electrical stimulation. The carcasses were chilled at 4 °C for 24 h, then the M. *longissimus thoracis* (LT) muscle was excised from the left side of the carcass between the 6th and the 13th rib and pH (pH_{24}) was measured on LT. The LT was vacuum packed and aged at 2 °C ± 1 °C until 48 h *post-mortem*. Then, a 3.5 cm thick sample was sliced from around the position of the 8th vertebra, vacuum packed and frozen at −18 °C until colour determination (colour at 48 h). The remaining section of the LT was vacuum packed and stored in the dark at 2 °C ± 1 °C until 10 days *post-mortem*. Then, one 3.5-cm thick sample at the 10th vertebra was sliced, vacuum packed and frozen at −18 °C for colour determination (colour at 10 days). Meat samples for colour determination from each country were transported on dry ice to the CREA-ZA (Italy).

Samples aged for 48 h and 10 days were thawed for 24 h, the bag was opened and the pH (pH_{10}) was

determined using a Hanna HI98240 pH-meter (Hanna Instruments Italia, Padova, Italy). A layer of superficial muscle was removed and each sample placed on a tray, overwrapped with film permeable to oxygen and maintained for one hour in the refrigerator at 4 °C to allow the myoglobin to bloom. Afterward, LT colour was measured using a Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings Inc.,Tokyo, Japan) in the CIELAB space (CIE, 1986) with a measured area diameter of 8 mm, including a specular component, 0% u.v. and a standard illuminant D65, which simulated daylight (colour temperature 6504 K) and an observer angle of 10°. The integrating sphere had a 52 mm diameter and the measurement area was covered with a CM-A149 dust cover. Zero and white calibrations were made with the cover. The lightness (L*), redness (a*) and yellowness (b*) were recorded, and the hue angle (h_{ab}) and chroma (C^*_{ab}) indexes were calculated as $C^*_{ab} = \sqrt{(a*)^2 + (b*)^2}$ and $h_{ab} = \tan^{-1}\left(\frac{b*}{a*}\right) \cdot \frac{180^\circ}{\pi}$. Reflectance spectra were recorded from 360 to 740 nm. The colour differences between two breeds (ΔE*) were calculated as $\Delta E^* = \sqrt[2]{(L^*_{xt} - L^*_{yt})^2 + (a^*_{xt} - a^*_{yt})^2 + (b^*_{xt} - b^*_{yt})^2}$, where 'x' and 'y' are the different breeds and 't' is the ageing time (48 h or 10 days). The relative percentage of metmyoglobin were calculated as:

$$\%\mathrm{MMb} = \left\{1.395 - \left[\frac{\langle A572 - A730\rangle}{\langle A525 - A730\rangle}\right]\right\} \cdot 100$$

where $A = \log\frac{1}{R}$. R is the reflectance at specific wavelength expressed as a decimal. The C^*_{ab}, h_{ab}, ΔE* and %MMb were calculated following the calculations detailed in AMSA (2012).

Statistical analysis

The pH_{10} values were analysed using the GLM procedure, with breed as fixed effect and the Bonferroni multiple-comparison procedure at $\alpha = 0.01$ was used to test significance of differences among breeds. As colour variation at 48 h and at 10 days were closely correlated, a GLM procedure was carried out with the five colorimetric variables (L*, a*, b*, C^*_{ab}, h_{ab}) measured at 48 h only, with breed as fixed effect and using the pH_{10} as covariate. Differences among least squares means were evaluated with the pdiff option with $\alpha = 0.01$. To study relationships between pH and colour variables, Pearson correlation coefficients between colour variables and pH_{24}, pH_{48} and pH_{10} were determined. In addition, a principal components analysis (PCA) was performed using L*, C^*_{ab}, h_{ab}, pH_{24} and pH_{10}. A VARIMAX rotation was applied to the retained components to redistribute the variance among factors to obtain factor pattern coefficients. The inclusion of pH_{24} and pH_{10} was checked, either separately or both together. When the pH at both times was included, the pH_{10} was retained in the second factor, negatively, and in the third factor, positively. However, pH_{24} was not retained in the factors with eigenvalues >1. Therefore, pH_{10} was used as it explained a higher percentage of variance.

A hierarchical cluster analysis (Ward's method for aggregation and Euclidian distance) using the matrix of ΔE* at 48 h and 10 days was performed to identify homogeneous groups of breeds. The clusters were established to maximise the intergroup variability and minimise the intragroup variability. The inter- and intra-class variabilities of the clusters of meat colour at 48 h were 83.4% and 16.67%, respectively. However, when the colour at 10 days was used the inter- and intra-class variability of the clusters were 74.2% and 25.8%, respectively. As the inter-class variability was greater, and the intra-class lower, for the clusters using colour at 48 h than at 10 days, only the dendrogram of colour similarity at 48 h between breeds was drawn. In addition, the five colorimetric variables (L*, a*, b*, C^*_{ab}, h_{ab}) of meat aged for 48 h were analysed with the GLM procedure with the cluster as fixed effect using the Bonferroni test with $\alpha = 0.01$ to compare means. Statistical analyses were carried out using the SAS statistical package v.9.3 software (SAS Institute Inc., Cary, NC, USA) except for the cluster analysis, which was carried out using the XLSTAT statistical package v.3.05 (Addinsoft, New York, NY, USA).

Results and discussion

Considerable variation was observed among the fifteen breeds for slaughter weight (Table 1). As it was studied by Albertí *et al.* (2008), this study underlines the large differences seen between dairy breeds and specialised beef breeds as well among local breeds, which were reflected in the studied traits. Regarding the pH, the pH_{24} was associated with the breed and ranged from an average of 5.57–5.82. Values of pH_{24} for each breed are available on Christensen *et al.* (2011). On average, pH_{10} was 0.06 units lower than pH_{24} and both pH values were correlated with each other ($r = 0.43$; $P < 0.0001$). Average values of pH_{10} (Table 1) ranged from 5.51 to 5.69 which are in the expected range for beef. These results are in accordance with average values reported previously, for example, 5.53 for Charolais (Renand *et al.*, 2001), 5.57 for Simmental and 5.54 for Angus (Chambaz *et al.*, 2003), 5.63 for Holstein (Barahona *et al.*, 2016) and an overall mean of 5.67 for carcasses of different cattle breeds, class and sexes in the Spanish market (Mach *et al.*, 2008).

The pH_{10} differed significantly among breeds ($P < 0.001$). The animal temperament and the management prior to slaughter are known to affect stress and

energy reserves in the meat that will affect ultimate pH (Miranda-de la Lama *et al.*, 2013). Differences in genetic susceptibility to stress among breeds are well known (Miranda-de la Lama *et al.*, 2013). In particular, animals with muscular hypertrophy having a more excitable temperament and are more prone to stress than non-hypertrophic animals (Oliván *et al.*, 2004). A negative correlation between pH at 48 h *post-mortem* and EUROP conformation score has been reported (Klont *et al.*, 1999). Values of conformation for each breed are available on Albertí *et al.* (2008). In the present study, a Pearson correlation of $r = -0.27$ ($P < 0.001$) was found between conformation score (Conformation score of the fifteen breeds were reported by Albertí *et al.* (2008)). and pH_{10}, which may partially explain pH differences between breeds. Ambient temperature influences the rate of pH fall (Maher *et al.*, 2004), which depends on abattoir cold room settings (Klont *et al.*, 1999). As experimental animals were slaughtered in different slaughterhouses, the ambient temperatures may have differed and in some cases ultimate pH may not have been reached in 48 h. The intra-breed variation was largest in Simmental, Holstein, Highland and Jersey than in the other breeds. On average, standard error of pH values reported in the present paper was lower than those reported some other authors (Renand *et al.*, 2001; Maher *et al.*, 2004) but are similar to those for animals raised and slaughtered under controlled conditions (Chambaz *et al.*, 2003; Serra *et al.*, 2004). Intra-breed variability is of interest for breeders who are seeking a recognisable brand, as they have to offer a homogeneous product to the market (Panea *et al.*, 2008). Regarding the relation between pH and colour, a negative correlation was found between both pH_{24} and pH_{10} with colour traits at 48 h and 10 days (Table 2), with pH_{10} having higher coefficients of correlation than pH_{24}. It is known that ultimate pH is not reached in the lighter carcasses by 24 h *post-mortem*.

Table 2 Pearson correlation coefficient and p values between pH at 24 h or pH at 10 days and colour variables from fifteen European young bulls breeds

	pH 24 h		pH 10 days	
	Coefficient	***P*-value**	**Coefficient**	***P*-value**
Colour at 48 h				
L*	−0.212	0.0001	−0.461	0.0001
a*	−0.091	0.06	−0.192	0.0001
b*	−0.246	0.0001	−0.480	0.0001
C^*_{ab}	−0.174	0.0003	−0.355	0.0001
h_{ab}	−0.108	0.03	−0.208	0.0001
%MMb	−0.023	0.64	−0.136	0.005
Colour at 10 days				
L*	−0.260	0.0001	−0.461	0.0001
a*	0.019	0.69	−0.228	0.0001
b*	−0.187	0.0001	−0.510	0.0001
C^*_{ab}	−0.071	0.15	−0.385	0.0001
h_{ab}	−0.193	0.0001	−0.202	0.0001
%MMb*	−0.007	0.89	0.002	0.96

*%MMb, Percentage of metmyoglobin.

Colour variables: principal component analysis, means and clustering

The principal component analysis is shown in Fig. 1. The first factor accounted for 47.2% of the variance and was explained mainly by lightness and h_{ab} values at 48 h and 10 days. The breeds (South Devon, Aberdeen Angus, Danish Red, Limousin, Asturiana de los Valles, Charolais, Piemontese and Marchigiana) had lighter and paler meat compared with the other breeds found towards the left side. The second factor accounted for 28.1% of the variance and was explained negatively by pH_{10} and positively by C^*_{ab}. Jersey, Simmental, Holstein and Highland breeds had the highest pH values and were found close together (pH_{10} on axis 2 in Fig. 1) while Marchigiana and Piemontese breeds, which had low pH values formed a separate group (opposite side of Fig. 1). Pirenaica and Holstein were projected at opposite positions on axis 2 because the greater C^*_{ab} of Pirenaica than Holstein. Highland, Simmental, Holstein, Aberdeen Angus, Red Danish and South Devon had lower C^*_{ab} values than the other breeds. Beef with lightness values >38 are bright, whereas beef with C^*_{ab} around 20 have a vivid red colour (MacDougall, 1982). Casina, Avileña-negra Ibérica, Pirenaica, Asturiana de los Valles, Marchigiana, Piemontese, Charolais and Limousin, which are found on the upper part of the biplot had a more vivid colour ($C^*_{ab} > 20$) while Highland, Simmental, Holstein, Aberdeen Angus, Red Cattle, Jersey and South Devon, at the lower part of the plot had a dull colour ($C^*_{ab} < 20$). Overall the meat of the breeds studied ranged between pale to dark red in colour (Albertí *et al.*, 2017).

Colour variables at 48 h and 10 days are highly correlated ($P < 0.0001$), with highest correlation coefficients for lightness ($r = 0.77$) and h_{ab} ($r = 0.73$), and medium correlation coefficients for C^*_{ab} ($r = 0.42$), redness ($r = 0.48$), yellowness ($r = 0.54$) and metmyoglobin percentage ($r = 0.48$). In consequence, Table 3 shows values for colour variables measured at 48 h post-mortem only. Values for all variables are in the range reported by other authors (Oliván *et al.*, 2004; Serra *et al.*, 2004). Intra-breed variability was, in general, similar to those reported by Chambaz *et al.* (2003) (SE = 0.4, in average) or Serra *et al.* (2004) (SE = 0.4, in average). Panea *et al.* (2008) reported that the L* of the muscle of Pirenaica was less variable than a* or b*, and muscle b* had a coefficient of variation that was nearly twice that of a*.

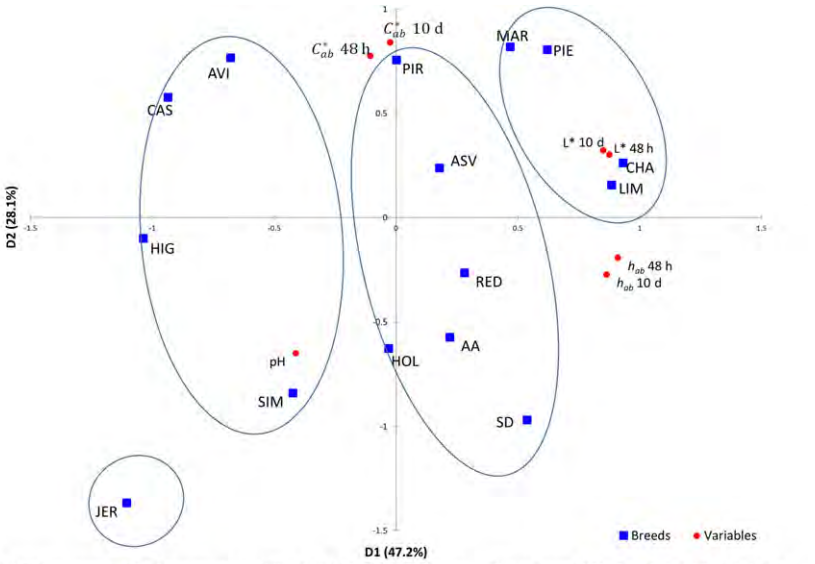

Figure 1 Biplot of the principal component analysis of colour and pH from fifteen European young bulls breeds.

AA: Aberdeen Angus; ASV: Asturiana de los Valles; AVI: Avileña; CAS: Casina; CHA: Charolais; RED: Danish Red; HIG: Highland; HOL: Holstein; JER: Jersey; LIM: Limousin; MAR: Marchigiana; PIE: Piemontese; PIR: Pirenaica; SIM: Simmental; SD: South Devon; L*: lightness; C^*_{ab}: chroma; h_{ab}: hue angle; pH: pH at 10 days.

Breed affected all colour traits (Table 3). Muscle colour at 48 h of Jersey, Highland, Casina, Simmental, and Avileña-Negra Ibérica breeds had lower L* and h_{ab} than Charolais, Piemontese, Limousin, and Marchigiana. South Devon and Holstein had the lowest metmyoglobin percentage (≤25%) while Charolais, Avileña and Casina presented the highest (≥31%). Breed effect on some colour variables have been widely described in literature, especially for L* (Gil *et al.*, 2001; Oliván *et al.*, 2004), but also for both L* and a* (Cuvelier *et al.*, 2006). Differences in L* between Angus, Simmental, Charolais and Limousin steers which were slaughtered at the same intramuscular fat content have been reported (Chambaz *et al.*, 2003). These authors concluded that when animals were slaughtered at the same percentage of adult live weight, differences between breeds were even higher than when animals were slaughtered at the same age, given that some breeds are early- and others late-maturing. In the present study, all the animals were slaughtered at approximately the same percentage of mature live weight to minimise differences due to

Table 3 M. *longissimus thoracis* colour and percentage of metmyoglobin of meat aged 48 h from fifteen European young bulls breeds

Breed	L*	a*	b*	C^*_{ab}	h_{ab}	%MMb
Aberdeen Angus	39.8 ± 0.46^{cd}	15.3 ± 0.40^{cde}	14.3 ± 0.25^{a}	21.0 ± 0.40^{ab}	43.6 ± 0.67^{bcde}	27.3 ± 0.54^{f}
Asturiana de los Valles	40.4 ± 0.46^{c}	14.5 ± 0.39^{defg}	13.6 ± 0.24^{abc}	20.0 ± 0.40^{bcd}	43.1 ± 0.67^{cdef}	28.4 ± 0.54^{def}
Avileña-Negra Ibérica	38.2 ± 0.46^{de}	16.4 ± 0.39^{abc}	13.3 ± 0.24^{c}	$21.1 \pm 0.4.0^{ab}$	38.8 ± 0.66^{gh}	31.2 ± 0.54^{ab}
Casina	37.3 ± 0.45^{e}	17.1 ± 0.39^{a}	$13.10.24^{c}$	$21.60.39^{ab}$	37.5 ± 0.65^{h}	31.0 ± 0.53^{ab}
Charolais	42.8 ± 0.45^{a}	13.3 ± 0.39^{i}	13.8 ± 0.24^{abc}	19.2 ± 0.39^{d}	46.2 ± 0.65^{a}	31.8 ± 0.53^{a}
Danish Red	40.5 ± 0.47^{bc}	13.9 ± 0.40^{efg}	13.3 ± 0.25^{bcde}	19.3 ± 0.41^{d}	44.1 ± 0.67^{abcd}	27.9 ± 0.54^{def}
Highland	36.9 ± 0.48^{e}	16.9 ± 0.41^{ab}	13.5 ± 0.25^{abc}	21.7 ± 0.41^{a}	38.5 ± 0.68^{gh}	29.4 ± 0.55^{bcde}
Holstein	39.6 ± 0.47^{cd}	14.8 ± 0.40^{defg}	13.7 ± 0.25^{abc}	20.2 ± 0.41^{bcd}	43.1 ± 0.68^{cdef}	25.0 ± 0.55^{g}
Jersey	34.4 ± 0.47^{f}	13.5 ± 0.40^{f}	10.5 ± 0.25^{d}	17.1 ± 0.41^{e}	37.7 ± 0.68^{h}	29.9 ± 0.55^{bcd}
Limousin	42.5 ± 0.46^{a}	13.6 ± 0.39^{fg}	14.0 ± 0.24^{abc}	19.5 ± 0.40^{cd}	45.8 ± 0.66^{abc}	30.8 ± 0.53^{abc}
Marchigiana	41.2 ± 0.49^{abc}	15.6 ± 0.42^{bcd}	13.8 ± 0.26^{abc}	20.8 ± 0.42^{abc}	41.5 ± 0.70^{efd}	28.5 ± 0.57^{def}
Piemontese	42.2 ± 0.48^{ab}	15.5 ± 0.41^{bcd}	14.2 ± 0.25^{ab}	21.1 ± 0.41^{ab}	42.5 ± 0.69^{def}	29.0 ± 0.55^{cdef}
Pirenaica	39.6 ± 0.46^{cd}	15.8 ± 0.39^{abcd}	13.2 ± 0.24^{abc}	21.0 ± 0.40^{ab}	41.0 ± 0.66^{fg}	29.7 ± 0.53^{bcde}
Simmental	37.9 ± 0.58^{de}	15.0 ± 0.50^{acdef}	13.0 ± 0.31^{c}	19.9 ± 0.51^{bcd}	41.2 ± 0.84^{efg}	27.6 ± 0.68^{ef}
South Devon	39.8 ± 0.49^{cd}	13.6 ± 0.41^{gh}	13.7 ± 0.26^{abc}	19.4 ± 0.42^{cd}	45.3 ± 0.70^{abc}	24.7 ± 0.56^{g}

Least square means ± standard error. Data were analysed by variance analysis with pH at 10 days as covariate.
Different lowercase letter in the same column implies statistical differences between breeds ($P < 0.05$).

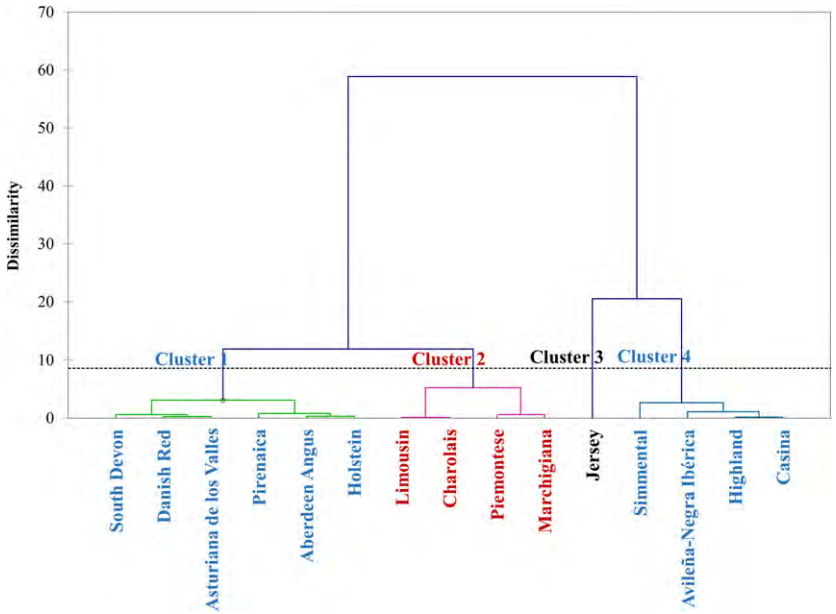

Figure 2 Hierarchical cluster analysis using the difference of colour (ΔE*) at 48 h of cattle breeds.

	L*	a*	b*	C^*_{ab}	h_{ab}
Cluster 1	39.9 ± 0.21^{b}	14.7 ± 0.18^{b}	13.7 ± 0.11^{ab}	20.2 ± 0.18^{a}	43.3 ± 0.33^{a}
Cluster 2	42.6 ± 0.24^{a}	14.7 ± 0.19^{b}	14.3 ± 0.1^{a}	20.5 ± 0.18^{a}	44.4 ± 0.34^{a}
Cluster 3	33.7 ± 0.38^{d}	13.1 ± 0.42^{c}	10.0 ± 0.33^{c}	16.4 ± 0.52^{b}	37.3 ± 0.46^{b}
Cluster 4	37.3 ± 0.23^{c}	16.4 ± 0.23^{a}	13.0 ± 0.14^{b}	20.9 ± 0.24^{a}	38.7 ± 0.32^{b}

Breeds comprised in each cluster: (1) South Devon, Danish Red, Asturiana de los Valles, Pirenaica, Aberdeen Angus and Holstein breeds; (2) Limousin, Charolais, Piemontese and Marchigiana; (3) Jersey; (4) Simmental, Avileña-Negra Ibérica, Highland and Casina.
Different lowercase letter in the same column implies statistical differences between breeds ($P < 0.05$).

Table 4 Differences between clusters in colour traits of *longissimus thoracis* muscle at 48 h *post-mortem*. Least square means ± standard error are shown for four identified breed-clusters

growth rates (Kempster *et al.*, 1982; Warris, 2000). Figure 2 shows the hierarchical cluster analysis using the difference of colour (ΔE*) at 48 h. The difference of colour measured grouped Jersey, Simmental, Avileña-Negra Ibérica, Highland and Casina together (Fig. 2) and the rest of the breeds formed a second group. Inside the first group, Jersey differed slightly from other breeds. The second group can be split into two subgroups with: Limousin, Charolais, Piemontese and Marchigiana in one, and the rest of the breeds in the other. To summarise, four clusters can be clearly defined according the similarity or dissimilarity of colour with one to six breeds in each (Table 4). Breeds could be clusters based on five colour traits and comprised (1) South Devon, Danish Red, Asturiana de los Valles, Pirenaica, Aberdeen Angus and Holstein breeds; (2) Limousin, Charolais, Piemontese and Marchigiana; (3) Jersey and (4) Simmental, Avileña-Negra Ibérica, Highland and Casina, with significant differences ($P < 0.0001$) between clusters. Cluster 3 was characterised by the lowest values of the five variables while Cluster 2 had the highest values of L*. Cluster 1 and 4 had intermediate values but Cluster 1 had higher values for L* and h_{ab} than Cluster 4. Therefore, the meat of these clusters could be defined as 'dark and dull red', 'bright and pale', 'red' and 'very bright and pale-red' for clusters 3, 1, 4 and 2, respectively. We want to point out that names of these clusters are based strictly in the colorimetric variables (Paterson, 2004).

Meat colour can be influenced by intramuscular fat content, as well as fibre type (Cuvelier *et al.*, 2006), which are both affected by muscle development (Bernard *et al.*, 2009; Hocquette *et al.*, 2012). In general, the higher the muscularity, the paler the meat colour and lower the intramuscular fat content. This is especially true for double-muscled animals, which carry a mutation in their myostatin gene, and are

characterised by a higher proportion of white muscle fibres (Fiems *et al*., 2003) and consequently, higher muscle glycolytic activity than normal animals (Gil *et al*., 2001; Oliván *et al*., 2004). However, differences in meat colour are also observed between different genetic types, which do not have a mutation in the myostatin gene. Divergent genetic selection for muscle growth potential e.g. between meat, dairy and dual purpose breeds also results in differences in muscle fibre types and IMF content (Hocquette *et al*., 2012). This is in part due to differentially expressed genes associated either with muscle mass or fat deposition in the carcass (Bernard *et al*., 2009).

Specialised beef breeds are generally late-maturing and fatten later than unimproved and dairy breeds (Albertí *et al*., 2005; Boligon *et al*., 2016). However, the variation in meat colour observed the present study is not related to fatness as assessed by intramuscular fat deposition (Christensen *et al*., 2011) or the dissected rib fat content (Albertí *et al*., 2008). Indeed, Piemontese and Charolais have a pale-red colour but Piemontese was the leanest breed and Charolais had intermediate levels of fat. Furthermore, Holstein, Danish Red, Aberdeen Angus and South Devon breeds had bright-red meat colour with a relative high fat levels, while Pirenaica and Asturiana de los Valles breeds with low fat depots also had bright red meat. If breeds are grouped according to body size and carcass traits (Albertí *et al*., 2008), the Piemontese, Asturiana de los Valles, Pirenaica, Limousin, South Devon, Charolais and Aberdeen Angus can be considered as specialised beef breeds, Avileña, Marchigiana and Simmental as intermediate or dual purpose breeds and Casina, Highland, Danish Red, Jersey and Holstein as unimproved and dairy breeds. Classification of breeds by colour or carcass traits gives quite similar groupings. In general, the specialised beef breeds have pale-red to bright-red lean meat colour but the Marchigiana had a pale-red colour while the Holstein and Danish Red had a bright-red colour. Muscle structure and fibres of the experimental animals have previously been characterised by their metabolic and contractile properties (Hocquette *et al*., 2007). These analyses revealed significant differences among breeds. Generally, dairy breeds and unimproved breeds had a high muscle oxidative metabolism as shown by high cytochrome-*c* oxidase (COX), citrate synthase (CS) or isocitrate dehydrogenase (ICDH) activities, which are generally associated with a high proportion of myosin heavy chain (MyHC)-I. High muscling and lean breeds, however, had the highest proportions of myosin heavy chain (MyHC) fast-glycolytic fibres (IIX) and the most glycolytic metabolism, as indicated by lactate dehydrogenase (LDH) activity. Usually the oxidative fibre characteristics (MyHC-I, ICDH, COX and CS) are negatively associated with glycolytic characteristics (MyHC-IIx and LDH) (Gagaoua *et al*., 2016). The red colour meat of Avileña and Casina and dark red of Jersey may be related to a high COX activity in muscle fibres (Cuvelier *et al*., 2006), in contrast Piemontese, with a high glycolytic metabolism, had a pale meat colour. These results are in agreement with the observations of other authors: Cuvelier *et al*. (2006) found that Aberdeen Angus had a low L* value associated with a low LDH metabolic activity and Blue Belgian bulls had light and pale meat due to their low mitochondrial enzyme activity (COX), whereas Limousin had intermediate characteristics. The light pale meat colour of the meat from Asturiana and Pirenaica may be related to a high proportion of IIA muscle fibres, of the fast-twitch oxidative-glycolytic type, and high glycolytic capacity (LDH/ICDH ratio) (Gil *et al*. (2001).

Genetic variation is likely to contribute substantially to animal-to-animal variation in lean meat colour (King *et al*., 2010). Molecular markers for meat quality have been analysed for the fifteen breeds studied here (Dunner *et al*., 2013). Two single nucleotide polymorphism (SNP) in PGAM2 were associated with L* and b*. PGAM2 catalyses the internal transfer of a phosphate group in the glycolysis process and it affects the activity of COX in muscle. Simmental, Limousin and Charolais have the highest frequency of the PGAM2 SNP associated with high COX activity (allele frequency: 1.00, 0.95 and 0.94), respectively, while Holstein had the lowest frequency (0.56). A SNP in the myofibrillar protein vimentin (VIM) gene, was associated with L* and b* at 10 days (Dunner *et al*., 2013). Limousin and Pirenaica has the same highest frequency of the VIM SNP (0.88) while Holstein and Jersey had the lowest frequency (0.37 and 0.34, respectively) of the same SNP. Therefore, the differences found in the colour of the meat between breeds could have a genetic basis associated with contractile and metabolic characteristics of muscle fibres.

Conclusions

The present study shows that lean meat colour differs significantly between the fifteen European cattle breeds investigated. These breeds can be grouped according to four scales of commercial interest: very bright and pale-red (Limousin, Charolais, Piemontese and Marchigiana), bright and pale (South Devon, Danish Red, Asturiana de los Valles, Pirenaica, Aberdeen Angus and Holstein), red (Simmental, Avileña-Negra Ibérica, Highland and Casina) and dark and dull red (Jersey). The differentiation between groups approximately correlate with body size and carcass traits, particularly fatness and muscle development and structure, and hence selection history of the breeds.

Therefore, the purpose of the breeds is related to the colour of the meat. The most specialised beef breeds had very bright and pale-red. Another beef breeds together with some dairy breeds had bright and pale meat. The intermediate beef breeds and dual-purpose breeds had red meat, and Jersey as a small dairy cattle had dull red meat.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

All procedures were approved by the in-house Ethics Committee for Animal Experiments of each participating research centres.

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